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RESEARCH ARTICLE

DETERMINATION OF PESTICIDE RESIDUES IN FRUITS LOCAL MARKET NANDED, INDIA

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Abstract

A study was conducted for the determination of pesticide residues in three fruits namely apple grapes and guava. Three pesticides including two organophosphates and one synthetic pyrethroid pesticides were analyzed. A total number of 36 samples of the collected from the local market of Nanded, India. The fruits samples were collected in the season basis on regular interval. The fruits were extracted by the application of a single phase extraction of 15 g of sample with acetonitrile containing 1% of acetic acid, followed by a liquid-liquid partition formed by the addition of MgSO4 and NaOAc. Cleanup of the extract was carried out with primary secondary amine (PSA) and magnesium sulphate. The quantification was done by GC-MS. The recovery varies from 75.0% to 105.0% with relative standard deviation (RSD) below 10%. However the limit of detection ranged from 0.001-1.00 mg kg^{-1}. Some of the fruit samples showed the presence of residues with one or other group of pesticides. Three pesticides were detected from total 36 samples with the range of 0.001-0.021 mg kg^{-1}. This method offers cheaper and safer alternative to typical multiresidue analysis methods for the fruits.
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Introduction

Fruits are an essential component of our diet due to their nutritional value. According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased[1]. As several insect pests attack the fruits, they are produced under very high input pressure. For better yield and quality, insecticides are applied during the entire period of growth and sometimes even at the fruiting stage. These pesticides are absorbed by the fruits and vegetables on consumption by human beings; it may be hazardous if safe waiting period is not adopted. Application of pesticides in modern agriculture has boosted farm productivity[2]. Pesticides contamination is a worldwide public health concern and also a main international trade problem[3]. Several pesticides are noxious substances and can persistent in the environment for a long time. Therefore, health point of view it is necessary to control the application of pesticides on crops[4,5]. However, levels of pesticides should be controlled at optimum point due to their relative toxicity to the environment and human health[6]. Thus, maximum residue levels (MRLs) for pesticides have established worldwide, which usually guide to manage the quantity of pesticides in foodstuffs. Residues resulting from the inappropriate use of pesticides on fruits have turn out to be most important concern in many countries, as well as in India. Currently India is the largest producer of pesticides in Asia and the third largest consumer of pesticides in the world. Thus the determination of pesticide residues in fruits and vegetables and other environmental components / commodities like soil, water and total diet have become increasingly essential requirements for consumers, producers and authorities for food quality control.

The short as well as long term impacts of the use of pesticides on biological systems are being evaluated continuously in an effort to minimize the hazards. A wide spread use, pesticides, their toxic residues have been reported in various environmental matrices by the researchers. [7,8,9]

Many researchers have estimated the pesticide residues (PRs) in various fruits including banana, mango, apple, peach, watermelon, melon, grape, orange, lemon, pear, pineapple, strawberry, raspberry, kiwi fruit, beet, papaya and litchi, etc[10-16] were reported the occurrence of pesticide residues to be even more than maximum residue level (MRL) values recommended by European union (EU), world health organization (WHO) and food and agricultural organization (FAO). The Department of Agriculture and cooperation, Ministry of Agriculture is running a central sector scheme, "Monitoring of Pesticide Residues at National Level" in food commodities and environmental samples. About 2062 fruits samples (apple, banana, grape, orange, pomegranate, guava and mango) samples were analysed by 14 laboratories in the year 2010-2011. Residues of mainly chlorpyrifos, cypermethrin and endosulfan were detected in 145 (7%) samples, pesticide residues were found above MRL in 20 (0.9%) samples only.[17]

Different extraction and quantification methods are used by various researchers for estimation of multi class pesticide residues in several vegetables and fruits. The main criteria for opting any methodology is that analytical method should be fast, easy, inexpensive and applicable to different matrices. In recent years, gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS)[18] and gas chromatography–tandem mass spectrometry (GC–MS/MS) [19].The variety of sensitive detectors coupled with GC such as electron capture detector (ECD)[20-22] nitrogen phosphorus detector (NPD)[23-25], pulsed flame photometric detector (GC-PFPD)[26] flame photometric detector (FPD)[27] improved the detection and quantification procedures of pesticide residues monitoring in different matrices.

A method of extraction, cleanup and determination of a pesticide residue by using gas chromatography (GC) equipped with mass detector (MS) for the separation, identification and quantification of chlorpyrifos, cypermethrin and monocrotophos on apple, grape and guava were developed and validated. Therefore, the purpose of this study was to develop an improved analytical method for the determination of chlorpyrifos, cypermethrin and monocrotophos on apples, grapes and guava samples collected from local market, Nanded, India.

EXPERIMENTAL

Chemical and apparatus

The organic solvent acetic acid, acetonitrile HPLC grade, magnesium sulphate and sodium acetate AR grade purchased from E Merck and primary secondary amine purchased from Agilent Technologies. The technical grade pesticide standards were used for standardizations and it were stored in a freezer. Anhydrous magnesium sulphate used during residue extraction was maintained at 300°C overnight and kept in air tight container. Polyethylene or PFTE 15ml and 50 ml with screw cap tubes.

Sample collection

A total of 36 **s**amples of fruits namely apples, grapes and guava were collected from the local market Nanded, India. The fresh samples of apple grape and guava collected in the year 2012 and 2013.

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Standard preparation

For preparation of stock solution, standards were dissolved in ethyl acetate and four levels of intermediate standard solution of each pesticide were prepared maintaining the same matrix concentration for the preparation of calibration curve and stored at -4°C in the dark. Working solutions were prepared daily by appropriate dilution with ethyl acetate.

Sample extraction and cleanup

The fresh apples, grapes and guava 2 kg each samples were taken for the extraction of pesticide residues. The samples were macerated to make paste with Philips mixer (equipped with stainless steel knives), a 15 g portion of the homogenized sample was weighed into a 50 ml polytetrafluoroethylene (PTFE) tube added 15 ml of acetonitrile containing 1% acetic acid (v/v). Then, 6 g MgSO4 and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added, and the sample was shaken forcefully for 4 min and kept in ice bath. The samples were then centrifuged at 4000 rpm for 5 min and 6 ml of the supernatant were transferred to a 15 ml PTFE tube to which 900 mg MgSO4 and 300 mg PSA were added. The extract was shaken using a vortex mixer for 20 s and centrifuged at 4000 rpm again for 5 min, approximately 2ml of the supernatant were taken in a vials. This extracts were evaporated to dryness under a stream of nitrogen and reconstituted in n-hexane in auto sampler tube for the GC-MS analysis.

Instrumentation

The analysis of pesticide was carried out by an integrated system of gas chromatography, equipped with automatic injection system and coupled to a mass spectrometric system with ion trap analyser. Varian CP-3800 GC, Saturn-2200 mass spectrometer with auto injector CP-8410 was used for analysis. The mass spectrometer was auto tuned using perfluorotributylamine (PFTBA). The separation of pesticide was done in a 30 meter length, 0.25 mm internal diameter and 0.25 μm film thickness coated with 5% phenyl-95% methylpolysiloxane Varian VF-5MS column. Helium was used as the carrier gas at 9.6 psi pressure and 1 ml min-1 flow. The injector was used at constant temperature 280^0C. The initial oven temperature was 80^0C (4min. isothermal) to 180^0C (at 20^0C min^{-1}) to 250^0C (at 2^0C min^{-1}) to 280^0C (at 10^0C min^{-1}) isothermal for 4 minutes. The injection volume was 1μL in splitless mode. The temperature of ion trap, manifold and transference line was 220^0C, 50^0C and 300^0C respectively. The mass spectrometer was used in SIM mode under electron impact at 70 eV and scan time 1 second. The computer that controlled the system also held a GC-MS library specially created for the target analytes under our experimental conditions. The mass spectrometer was calibrated weekly with perfluoro-tributylamine. Helium (99.999%) at a flow-rate of 1 ml min^{-1} was used as carrier and collision gas.

RESULTS AND DISCUSSION

Identification and confirmation of target analytes

The confirmation of a compound was done by comparing the GC–MS spectra obtained in the sample with stored as reference spectrum in the same experimental conditions. The reference spectra were obtained by injecting a blank apple, grape and guava sample spiked at the known concentration of the pesticides.

Limit of detection and limit of quantification

The limit of detection (LoD) was calculated from the peak intensity at 0.01mg kg^{-1} and blank in recovery tests. The LoD of chlorpyrifos cypermethrin and monocrotophos, was 0.004, 0.003, and 0.005 mg kg^{-1} respectively. LoQ was obtained for chlorpyrifos, cypermethrin and monocrotophos, was 0.012, 0.009 and 0.015 mg kg^{-1} respectively (table 1). Linear calibration curves were found between peak areas and analyte concentration in the whole range of studies. Quantitation ion, confirmation ion and calibration range of monocrotophos, chlorpyrifos and cypermethrin (table 2). The correlation coefficients of analytical curves were near 0.99, with linearity for each compound, which allows the quantitation of these compounds by the method external standardization.

Validation of the method

In order to check the feasibility of the GC-MS method for the analysis of pesticide residues in fresh sample extracts, it was validated using apple and grapes extracts.

Recovery

Recovery studies were performed to examine the efficacy of extraction and clean up. Untreated apples, grapes and guava samples were spiked with known concentration of the pure pesticides standard solution and extraction and clean-up were performed as described earlier. The concentration of each pesticide in the final extracts was calculated (table 3). The average recoveries of pesticide residues in apple, grape and guava samples were 75.0 to 105.0 %.

Application of analysis to the market samples

In order to test the feasibility of the GC–MS approach for routine analysis of pesticide residues in the market samples of fruits (apple, grape and guava) were analysed for the target compounds related on basis of their frequency of application. The concentrations of each pesticide were obtained and calculated in the final extracts of the vegetables samples collected from local market (table:4 to 6).

The results indicate the presence of pesticide residues. Maximum samples contained residues of pesticides while no samples were exceeded the MRLs. Samples of apple, grape and guava fruits showed detectable amounts of chlorpyrifos, cypermethrin and monocrotophos (0.001–0.021 $mgkg^{-1}$). In India monitored fruits, organochlorine pesticides i.e., HCH, DDT and endosulfan were detected in almost all the fruits samples of ber, grapes and guava.[28] Fruit samples of winter fruits (apple, grapes, banana cheeku, papaya, lemon) for pesticide residues employing a multiresidue analysis. All the fruit samples showed the presence of residues with one or other group of pesticide.[29]

4. CONCLUSION

The outcomes of the present study authenticate the existence of pesticides such as chlorpyrifos, cypermethrin and monocrotophos in fruit samples which were applied in pre-harvest treatment. To avoid adverse effects on public health it is a necessity to set up control measures so as to make sure that each pesticide should be below MRLs in the fruits to be marketed. On the bases of achieved results, it is recommended that regular evaluation of pesticide residue should be carried out on each fruit for the planning and future policy about the formulation of standards and quality control of pesticides. The objective of this study was to create awareness among the vegetables consumers who were consuming contaminated fruits. The developed processing method was reducing the residue to an acceptable and less harmful level. This method was very simple for removal of pesticide residue from the produce and consumer can apply this technique in their houses.

Table 1: Molecular formula, retention time, LODs and LOQs of monocrotophos, chlorpyrifos and cypermethrin.

Compound	Molecular formula	RT (min)	LoDs (mg kg^{-1})	LoQs (mg kg^{-1})
Monocrotophos	$C_7H_{14}NO_5P$	17.79	0.005	0.015
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	25.02	0.004	0.012
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	31.22	0.003	0.009

Table 2: Quantitation ion, confirmation ion and calibration range of monocrotophos, chlorpyrifos and cypermethrin.

Compound	Quantitation ion	Confirmation ion	Calibration range(mg kg^{-1})	Correlation coefficient	Coefficient of variation (n = 5) %
Monocrotophos	127	98	0.02-1.00	0.991	4.58
Chlorpyrifos	97	314	0.02-1.00	0.988	4.80
Cypermethrin	181	127	0.02-1.00	0.990	4.65

Table 3: Recovery of pesticides in the spiked samples.

Sample	Compound	Concentration (mg kg^{-1})	Recovery (%)	Coefficient of variation(n=5)%
Apple	Monocrotophos	1.0	102.00	4.58
Apple	Chlorpyrifos	1.0	99.80	4.78
Apple	Cypermethrin	1.0	98.60	4.80
Grape	Monocrotophos	1.0	105.00	4.85
Grape	Chlorpyrifos	1.0	99.40	4.80
Grape	Cypermethrin	1.0	98.30	4.64
Guava	Monocrotophos	1.0	88.97	4.60
Guava	Chlorpyrifos	1.0	97.02	4.60
Guava	Cypermethrin	1.0	96.05	4.98

Table 4: Amount of pesticide residues detected in apple samples collected from local market, Nanded, analysed by GC-MS using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Sample collection period	No. of sample collected	Chlorpyrifos (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Monocrotophos (mg kg^{-1})
July- 2013	1 1	0.008 ND	ND 0.001	ND ND
August -2013	1 1	ND 0.002	ND ND	ND ND
September-2013	1 1	ND ND	ND 0.004	0.04 ND
October-2013	1 1	ND 0.011	0.002 ND	ND ND
November-2013	1 1	ND 0.002	ND ND	0.007 ND
December-2013	1 1	0.001 ND	ND 0.002	ND ND

Table 5: Amount of pesticide residues detected in grape sample collected from local market, Nanded, analysed by GC-MS using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Sample collection period	No. of sample collected	Chlorpyrifos (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Monocrotophos (mg kg^{-1})
November-2012	1 1	ND 0.021	ND 0.001	ND ND
December-2012	1 1	ND 0.002	ND ND	0.004 ND
January-2013	1 1	ND 0.008	ND ND	ND ND
February-2013	1 1	ND 0.011	0.002 ND	ND ND
March-2013	1 1	ND ND	ND ND	0.005 ND
April-2013	1 1	0.016 ND	ND 0.002	ND ND

Table 6: Amount of pesticide residues detected in guava samples collected from local market, Nanded, analysed by GC-MS using 1% acetic acid solution of acetonitrile cleaned up with PSA and magnesium sulphate.

Sample collection period	Sample collected	Chlorpyrifos (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Monocrotophos (mg kg^{-1})
July-2012	1 1	ND 0.002	ND ND	ND ND
August-2012	1 1	ND ND	0.005 ND	ND ND
November-2012	1 1	ND 0.008	ND 0.004	0.012 ND
December-2012	1 1	ND 0.006	ND ND	0.005 ND
March-2013	1 1	ND ND	0.007 ND	ND ND
April-2013	1 1	ND ND	ND ND	0.002 ND

ND = Not Detected
MRL- Apple (Codex) 0.5 mg kg^{-1} for chlorpyrifos
Grape (Codex) 0.2 mg kg^{-1} for cypermethrin
Guava (Codex) 0.5 mg kg^{-1} for chlorpyrifos

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